

The Examiner rejected claims 18, 21, 23 and 25 because the genus and species of the microorganisms were not italicized or underlined. Appropriate correction of the claims have been made such that the genus and species of microorganisms are italicized.

The Examiner rejected claim 20 for having parenthesis around the "A" and "I" designations, and because "X" is undefined. The recitation of the parenthesis around the "A" and "I" meant possible alternatives to the "G" and "L" amino acids, respectively. Further, the abbreviation "X" is well known in the art as an abbreviation for any given amino acid. Although Applicants believe that one skilled in the art would understand the claim as it was originally submitted, Applicants have nonetheless amended the claim such that there can be no misunderstanding as to the meaning of the abbreviated terms. Applicants believe that this amendment does not alter the scope of the claims as they would be understood by one of skill in the art, and no surrender of scope of the claims is intended.

The Examiner rejected claim 22; however, claim 22 has been canceled and the rejection is obviated.

Rejections under 35 USC §112, first paragraph

The Examiner rejected all claims under 35 USC §112, first paragraph. The Examiner stated that the specification teaches that when the genes citC, citD, citE, citF, citX and citG are placed into an expression vector, with the citC, citD, citE, citF and citG genes being obtained from *Klebsiella pneumoniae* and the citX obtained from *E. coli*, and this vector transformed into *E. coli*, an active citrate lyase was produced.

The claims as amended are limited such that the plasmid contains the listed genes, and that at least four of the genes are from *Klebsiella pneumoniae* and the DNA fragment is obtainable from *E. coli*. Support for the claims as amended is found throughout the specification and the claims properly delineate that which is enabled by the specification. Withdrawal of this rejection is respectfully requested.

Rejections under 35 USC §102

Claims 16-17 and 22-26 stand rejected under 35 USC §102 as being anticipated by Bott et al. Applicants have amended the claims such that the limitations previously found in claim 18

are now found in claim 16. Claim 18 was not rejected under this statutory provision, and all the claims as amended should therefore stand. Withdrawal of this rejection is respectfully requested.

Rejections under 35 USC §103

Claims 16-22 and 24-26 were rejected under Rejections under 35 USC §103 over Blattner et al. or Oshima et al. The claims have been amended such that this rejection should be withdrawn. The invention is essentially based on the surprising fact that co-expression of *K. pneumoniae* genes in combination with a DNA fragment from *E. coli* results in an active form of holo-citrate lyase. Without the co-expression of an *E. coli* fragment, e.g., the citX gene, only inactive apo-citrate lyase is produced. The claims have been amended to reflect this invention.

Applicants agree with the Examiner in so far as the genome of *E. coli* contains the genes for citC, citD, citE, citF, citX and citG in that order. However, the expression of said *E. coli* gene cluster does not result in a soluble active citrate lyase in adequate yields. The desired result is only obtained by using the inventive method disclosed in the present application, particularly if the expression occurs under aerobic conditions. Moreover, neither of the cited references provide any teaching or indication which would induce a person skilled in the art to express the defined *K. pneumoniae/E.coli* gene cluster in order to obtain a protein with citrate lyase activity in an active form. For this reason, the claims as amended obviate the rejections based upon Rejections under 35 USC §103. Withdrawal of the rejections is respectfully requested.

For the reasons stated, Applicants believe the claims as amended are in condition for allowance, and respectfully request that the Examiner allow such claims to issue.

Respectfully submitted,



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Marked-up claims

16. A method for the production of a protein with citrate lyase activity, said method comprising the steps of expressing a suitable plasmid in a host organism and isolating the protein in an active form[.]; wherein the plasmid contains the genes citC, citD, citE, citF, citG and a DNA fragment obtainable from *E. coli* that is located between citF and citG on the *E. coli* citrate lyase gene cluster and an inducible promoter[.]; and wherein at least four genes are derived from *Klebsiella pneumoniae*.
17. The method of claim 16, wherein the genes code for certain subunits of the protein having citrate lyase activity and[/or] for components that contribute to the biosynthesis of the complete enzyme.
18. (Canceled)
19. The method of claim [18] 16, wherein one of the genes or the DNA fragment codes for a 20 kDa protein.
20. The method of claim [18] 16, wherein one of the genes or the DNA fragment codes for a protein containing the motif X₁-R-L-X₂-D-X₃-D-V, wherein X₁ is optionally G or A, X₂ is any amino acid, and X₃ is optionally L or I.
21. [Canceled]
22. [Canceled]
23. [Canceled]
24. The method of claim 16, wherein the host organism is a eukaryotic or prokaryotic microorganism.
25. The method of claim 24, wherein the host organism is *E. coli*.

Marked-up claims

26. The method of claim 16, wherein the expression occurs under aerobic conditions.